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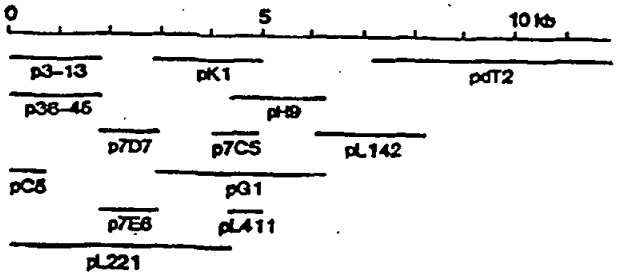
(54) Title: MAIZE CHLOROTIC DWARF VIRUS AND RESISTANCE THERETO

(57) Abstract

Methods and materials are provided to isolate the coat protein genes from maize chlorotic dwarf virus. One or more of these genes (MCDV-$CP_1$, MCDV-$CP_2$ or MCDV-$CP_3$) is then incorporated in an expression cassette designed for suitable expression in a plant cell system. The resulting transformation vector is then introduced into maize to provide cross protection to MCDV or related viral infections.



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# MAIZE CHLOROTIC DWARF VIRUS AND RESISTANCE THERETO

## Technical Field

This invention relates to providing plants with resistance to maize chlorotic dwarf virus (MCDV) and viruses to which MCDV infection or resistance provides cross-resistance, including maize dwarf mosaic virus strain A (MDMV-A).

## Background of the Invention

Virus-induced diseases in agronomically important crops have cost farmers a great loss of income due to reduced yields. Traditionally, virus diseases have been controlled by breeding for host plant resistance or by controlling insects that transmit diseases. Chemical means of protection are not generally possible for most viruses, and where possible are not generally practical. It has been known for many years that viral symptoms can be reduced in virus-infected plants by prior inoculation with a mild strain of the same virus, a phenomena known as cross-protection, as described by Sequeira, L., Trends in Biotechnology, 2, 25 (1984). Cross-protection is considered successful if the disease symptoms of the superinfecting (the more virulent) virus can be delayed or suppressed. There are several disadvantages to applying this type of cross-protection to the field situation:

1) application of the mild strain virus to entire fields is usually not practical,

2) the mild strain might undergo mutation to a more highly virulent strain,

3) the protecting strain might interact synergistically with a non-related virus causing a severe pathogenic infection,

4) a protecting virus in one crop may be a severe pathogen in another crop, and

5) a protective strain may cause a significant loss of yield in itself.

One proposed solution to these disadvantages has been to introduce a single viral gene into the host plant genome to cross-protect, rather than infect with an intact virus. This single gene cross-protection strategy has already been proven successful using the coat protein gene from tobacco mosaic virus (TMV-CP). As

reported by Abel, P.P., et.al., Science, 232, 738 (1986), transgenic tobacco plants, expressing TMV mRNA and coat protein (CP),demonstrated delayed or suppressed symptom development upon infection with TMV. TMV-CP transgenic tomato plants have been described by Nelson, R.S., et.al., Bio/Technology, 6, 403 (1988), to show evidence of protection from TMV as well as three strains of tomato mosaic virus (ToMV). Other approaches using DNA clones of viruses to engineer resistance include positive interference, as described by Golemboski et al. Proc. Natl. Acad. Sci. USA, 87, 6311 (1990) and Carr and Zaitlin, Mol. Pl. Microbe Inter., 4, 579 (1991); and antisense RNA, as described by Powell et al., Proc. Natl. Acad. Sci. USA, 86, 6949 (1989).

Numerous viruses exist for which resistance is desired. Maize chlorotic dwarf virus causes a somewhat variable mosaic or yellow streaking and occasional stunting in maize. Early infections can result in severe symptoms including premature death. The virus is spread by the blackfaced leafhopper (Graminella nigrifons). MCDV can overwinter in Johnsongrass (Sorghum halepense) and as a result has become a recurrent problem in areas where Johnsongrass is a common weed. Combined infections with maize dwarf mosaic virus can cause more severe symptoms although the syndrome is less well characterized than Corn Lethal Necrosis. Only limited success has been obtained to date in developing MCDV-resistant maize lines, due to the difficulties of selecting efficiently for resistance to an obligately insect transmitted virus, as well as a lack of usable sources of resistance in agronomically useful maize lines. Thus, there is a continuing need for genes, plant transformation vectors, and transformed plant materials providing resistances to pathogenic viruses such as MCDV.

Unfortunately, while certain plant viruses, such as tobacco mosaic virus, have coat protein genes that are found on subgenomic RNA and are therefore relatively easy to identify and clone for use in engineered cross-protection, maize chlorotic dwarf virus belongs to a completely separate group, the only other (tentatively assigned) member of which is the spherical virus of the rice tungro disease (RTSV). In addition, MCDV has a number of unusual biological properties which make identification of an appropriate gene difficult. For example, all attempts to mechanically transmit MCDV have been unsuccessful. As another example, MCDV appears to be a phloem-restricted virus. MCDV also has three coat proteins, and it was not known whether expression of one protein would be sufficient to confer immunity or whether all three would need to be expressed. Nor was it known

which protein would be the appropriate one to express if only one could be expressed. Further, the genome of MCDV has an unusual genome organization to provide for the expression of multiple coat proteins.

**Brief Description of the Drawing Figures**

Figure 1 is a schematic illustration of the manner in which the nucleic acid sequence of MCDV-type strain was obtained by sequencing overlapping cDNA clones.

Figure 2 is an a schematic illustration of the unusual organization of the MCDV genome.

**Disclosure of the Invention**

In the present invention, methods and materials are provided to isolate any or all of the three coat protein genes from maize chlorotic dwarf virus (MCDV). One or more of these genes (MCDV-$CP_x$, where x is 1, 2, or 3) is then incorporated in an expression cassette designed for suitable expression in a plant cell system. The resulting transformation vector is then introduced into maize callus to provide cross-protection to MCDV-related viral infections. MCDV has a single, long RNA core having the sequence shown in SEQUENCE I.D. No. 4.

Description of the Preferred Embodiments

The present invention provides cDNA clones from the RNA genome of maize chlorotic dwarf virus which code substantially solely for the coat protein of the virus. These clones are incorporated into an expression cassette in which the cDNA clone is operably linked to plant or bacterial regulatory sequences which cause the expression of the cDNA clone in living plant or bacterial cells, respectively. It is important that the cloned gene have a start codon in the correct reading frame for the structural sequence. The resulting bacterial vectors can be readily inserted into bacteria for expression and characterization of the sequence. Accordingly, the present invention also provides bacterial cells containing as a foreign plasmid at least one copy of the foregoing bacterial expression cassette. In addition, the plant expression cassette preferably includes a strong constitutive promoter sequence at one end to cause the gene to be transcribed at a high level and a poly-A recognition sequence at the other end for proper processing and transport of the messenger RNA. An example of such a preferred (empty) expression cassette into which the cDNA of the present invention can be inserted is the pPHI414 plasmid developed by Beach et al. of Pioneer Hi-Bred International, Inc., Johnston, IA, as disclosed in U.S. Patent Application No. 07/785,648, filed October 31, 1991. Highly preferred

plant expression cassettes will be designed to include one or more selectable marker genes, such as kanamycin resistance or herbicide tolerance genes. The plant expression vectors of this invention can be inserted, using any convenient technique, including electroporation (in protoplasts), microprojectile bombardment, and microinjection, into cells from monocotyledonous or dicotyledonous plants, in cell or tissue culture, to provide transformed plant cells containing as foreign DNA at least one copy of the DNA sequence of the plant expression cassette. Preferably, the monocotyledonous species will be selected from maize, sorghum, wheat and rice, and the dicotyledonous species will be selected from soybean, alfalfa, tobacco and tomato. Using known techniques, protoplasts can be regenerated and cell or tissue culture can be regenerated to form whole fertile plants which carry and express the desired cDNA clone for MCDV coat protein. Accordingly, a highly preferred embodiment of the present invention is a transformed maize plant, the cells of which contain as foreign DNA at least one copy of the DNA sequence of an expression cassette of this invention.

Finally, this invention provides methods of imparting resistance to maize chlorotic dwarf virus to plants of a MCDV susceptible taxon, comprising the steps of:

a) culturing cells or tissues from at least one plant from the taxon,

b) introducing into the cells of the cell culture or tissue culture at least one copy of an expression cassette comprising a cDNA clone from the RNA genome of MCDV which codes substantially solely for the coat protein of the virus, operably linked to plant regulatory sequences which cause the expression of the cDNA clone in the cells, and

c) regenerating MCDV-resistant whole plants from the cell or tissue culture. Once whole plants have been obtained, they can be sexually or clonally reproduced in such manner that at least one copy of the sequence provided by the expression cassette is present in the cells of progeny of the reproduction.

Alternatively, once a single transformed plant has been obtained by the foregoing recombinant DNA method, conventional plant breeding methods can be used to transfer the coat protein gene and associated regulatory sequence via crossing and backcrossing. Such intermediate methods will comprise the further steps of

a) sexually crossing the MCDV resistant plant with a plant from the MCDV susceptible taxon;

b) recovering reproductive material from the progeny of the cross; and

c) growing resistant plants from the reproductive material. Where desirable or necessary, the characteristics of the susceptible taxon can be substantially preserved by expanding this method to include the further steps of repetitively:

a) backcrossing the MCDV resistant progeny with MCDV susceptible plants from the susceptible taxon; and

b) selecting for expression of MCDV resistance among the progeny of the backcross,

until the desired percentage of the characteristics of the susceptible taxon are present in the progeny along with the gene imparting MCDV resistance.

By the term "taxon" herein is meant a unit of botanical classification of genus or lower. It thus includes genus, species, cultivars, varieties, variants, and other minor taxonomic groups which lack a consistent nomenclature.

It will also be appreciated by those of ordinary skill that the plant vectors provided herein can be incorporated into Agrobacterium tumefaciens or Agrobacterium rhizogenes, which can then be used to transfer the vector into susceptible plant cells, primarily from dicotyledonous species. Thus, this invention provides a method for imparting MCDV resistance in Agrobacterium-susceptible dicotyledonous plants in which the expression cassette is introduced into the cells by infecting the cells with Agrobacterium tumefaciens, a plasmid of which has been modified to include the plant expression cassette of this invention. The following description further exemplifies the compositions of this invention and the methods of making and using them. However, it will be understood that other methods, known by those of ordinary skill in the art to be equivalent, can also be employed.

1. Isolation and cloning of MCDV cDNA

The type strain of MCDV was maintained in the maize inbred Oh28 by transmission with the leafhopper G. nigrifrons and viral particles were isolated as previously described (Hunt et al., Phytopathology 78, 449 (1988)). MCDV particles were suspended in NETS (10 mM Tris, pH 7.5; 100 mM NaCl; 1 mM $Na_2EDTA$; 0.5% SDS) and extracted with 1:1 chloroform:phenol to isolate MCDV RNA.

First and second strand cDNA synthesis were by the method of Gubler and Hoffman, Gene 25, 263 (1983) utilizing cDNA synthesis kits (Amersham, Arlington Heights, IL). For the initial cDNA libraries, double-stranded cDNA was treated

with EcoRI methylase, ligated to GGAATTCC EcoRI linkers, digested with EcoRI and separated from linkers by column fractionation. The cDNA was ligated to EcoRI-cleaved _gt10 and EcoRI-cleaved, phosphatased (CIP) _gt11 phage arms. After packaging, the _gt10 phage were plated on bacterial strain NM514 and screened for MCDV-specific inserts by filter plaque hybridization (Benton and Davis, Science 196, 180 (1977)), using $^{32}$P-labeled cDNA's random-primed from the MCDV genomic RNA. MCDV-positive phage were purified and the cDNA inserts subcloned into pUC119 (Vieira and Messing, Meth. Enzymol. 153, 3 (1987)) for further analysis. Hybridization positive clones from the initial gt10 library included: p3-13, p36-45, pH9, pK1, pG1, pC5 (Figure 1). After packaging, the _gt11 phage were plated on bacterial strain Y1090$^{r-}$ and screened with antisera to either intact MCDV virions or isolated, individual MCDV capsid proteins (Maroon, MS Thesis, Ohio State University (1989)) as described by Mierendorf, et al. Meth. Enzymol. 152,458 (1987). Positive phage clones were identified with antisera specific to either cp1 or cp2, and cDNA inserts from these phage were subcloned into pUC119. The anti-cp1-specific cDNA clone, p7C5, and the anti-cp2-specific cDNA clones, p7E6 and p7D7, (Figure 1) were chosen for study. Analysis of initial cDNAs revealed that a number of clones terminated at identical EcoRI sites which were shown to be present in the viral sequence. This result indicated that the methylation of the initial cDNAs was incomplete. To obtain cDNAs to the rest of MCDV and to overlap the initial clones, two additional cDNA libraries were prepared, one primed with oligo-dT(12-18) and one random-primed. Double-stranded cDNA prepared as above was ligated to a 20/24 nt. blunt end/EcoRI adaptor (Amersham), and adaptor cDNAs were kinased and ligated to EcoRI-cleaved/phosphatased pUC119. Plasmid clone pdT2 (Figure 1) was derived from the dT-primed library and plasmids pL142, pL221, and pL411 (Figure 1) were derived from the random-primed library.

2. Sequencing of MCDV cDNA

Single-stranded DNA templates for sequencing were derived by superinfection with M13K07 of bacterial strain MV1190 containing the pUC119 based cDNAs (Figure 1), cloned in both orientations, as described by McMullen et al., Nuc. Acids Res., 14, 4953 (1986) and Vieira and Messing, Meth. Enzymol. 153, 3 (1987). Ordered deletions from the full-length single-stranded templates were prepared by the method of Dale et al., Plasmid 13, 31 (1985). Dideoxynucleotide sequencing reactions with the Klenow fragment of Pol. I or Sequenase (U.S.

Biochemicals, Cleveland, OH) were performed using $^{35}$S-dATP. Greater than 99% of the total sequence was obtained from both strands and the majority was read from three or more templates. The 5' sequence not contained on cDNA was obtained by direct RNA sequencing, using the sequencing primer 5'-GGTCTACTCACGGCACGCCA-3' (SEQUENCE I.D. NO. 3) with an RNA sequencing kit (Boehringer Mannhein, Indianapolis, IN) as recommended except that tailing of reaction products with dTTP by terminal deoxynucleotidyl transferase using the method of DeBorde et al., Anal. Biochem. 157,275 (1986) was added to improve resolution of final bases.

To obtain the amino-terminal protein sequence of MCDV capsid proteins, MCDV particles were disrupted in Laemmli loading buffer and the individual capsid protein separated on a 12.5%-4% Laemmli slab gel (Laemmli, Nature 227, 680 (1970)). The proteins were electrotransfered to Immobilon-P membrane (Millipore, Bedford, MA) using a 10 mM CAPS, pH 11.0; 10% MeOH transfer buffer, stained with Coomassie Blue R-250 for visualization and excised. Automated amino-terminal protein sequencing was performed by the Iowa State University Biochemistry Instrumentation Center (Ames, IA).

DNA and protein sequence analysis was performed using the IntelliGenetics (Mountain View, CA) molecular biology software on a Digital VAX 8250 located at the USDA-ARS-ASRR (Agricultural Systems Research Resource) Beltsville, MD.

The nucleic acid sequence of MCDV-type strain was obtained by sequencing overlapping cDNA clones (Figure 1) that covered all but 13 nucleotides at the 5' terminus of MCDV. The 5' end sequence was obtained by direct RNA sequencing. Despite repeated attempts and the use of terminal transferase in the manner of DeBorde et al., Anal. Biochem., 157, 275 (1986) the first nucleotide could not be definitely determined. In part for this reason, the expressions "coding substantially for" and "coding substantially solely for" are used herein, and with regard to the use of the word "substantially" refer to sequences which code for no more than a few (five or less) amino acids greater or lesser on either end of the desired protein or proteins, or which have an equivalent number of nucleotide bases more or less than the native sequence.

The genomic RNA of MCDV-type (SEQUENCE I.D. NO. 4) was determined to be 11785 nucleotides long, exclusive of the poly-A tail at the 3' terminus. This sequence permits the construction of a DNA molecule which codes for the entire maize chlorotic dwarf virus, or any portion or functional unit thereof which is

useful in conferring resistance to the virus when expressed in plant cells. Such resistance can readily be evaluated using routine testing methods such as those disclosed herein. Computer anlaysis of the sequence indicated a long open reading frame from nucleotide 456 to nucleotide 10826. The translation of this open reading frame would result in a protein of 3457 amino acids with a derived molecular weight of 388,890 daltons. The open reading frame begins with two AUG triplets, neither of which is in a particularly favorable context for initiation of translation when compared with the analyses of translation start sequences by Lutcke et al., EMBO J. 6, 43 (1987); and Kosak, J. Cell Biol. 108, 229 (1989) by the scanning model. In addition, there are 13 AUG triplets preceding the double AUG that starts the open reading frame. A long untranslated 3' leader containing multiple AUG triplets before the beginning of a very long reading frame is similar to the animal picornaviruses as described by Stanway, J. Gen. Virol. 71, 2483 (1990). Internal initiation at the AUG for the long open reading frame has been demonstrated to occur for a number of the animal picornaviruses as seen in Pelletier and Sonenberg, Nature, 334, 320 (1988) and Jang et al., J. Virol., 63, 1651 (1989). The mechanism for initiation of translation for MCDV has not been characterized.

The derived amino acid sequence of MCDV-type was compared to the Protein Identification Resource, Version 32 and the University of Geneva, Version 22, protein data banks for sequence similarity using the IFIND (IntelliGenetics) program based on the algorithm of Wilber and Lipman, Proc. Natl. Acad. Sci. USA, 80, 726 (1983). The highest similarity score was with the comovirus, cowpea mosaic virus (CPMV) as reported by Lomonossoff and Shanks, EMBO J., 2, 2253 (1983) and the second highest score was with the nepovirus, grapevine fanleaf virus (GFLV) as reported by Ritzenthaler et al., J. Gen. Virol., 72, 2357 (1991). For both viruses the region of similarity preceeded and included the first conserved motif of RNA-dependent RNA polymerases as defined by Poch et al. EMBO J., 8, 3867 (1989). The IFIND program identified weaker similarity with additional nepoviruses and some of the animal picornaviruses. The conservation of protein sequence and gene order for the plant comoviruses, nepoviruses and potyviruses, and the animal picornaviruses is well documented by, inter alia, Agros et al., Nuc. Acids Res., 12, 7251 (1984); Goldbach, Ann. Rev. Phytopath., 24, 289 (1986); and Domier et al., Virology, 158, 20 (1987) and has led to the proposal of the picornavirus-like "supergroup". Two additional conserved protein regions involved in genome replication for picorna-like viruses are the NTP binding/helicase region,

as described by Agros et al., above, and Gorbalenya et al., Nuc. Acids Res., 17, 4713 (1989) and the C-terminal region, cysteine active site of the 3C-like proteases, as also described by Agros et al., above, and by Grief et al., J. Gen. Virol., 69, 1517 (1988).

The electrophoresis of MCDV virions on denaturing protein gels reveals three structural proteins, designated cp1, cp2 and cp3 with molecular weights of 32.5 kd, 27 kd, and 24.5 kd; respectively. Antiserum specific to cp1 was used to screen a _gt11 library to isolate the clone p7C5, and antiserum specific to cp2 was used to identify the cDNAs p7E6 and p7D7 (Figure 1). This result indicated that an antigenic region of cp1 was located between 4063-4903 and an antigenic region of cp2 was located between 1815-2941. Automated amino-terminal sequencing was performed on each of the MCDV capsid proteins. The amino-terminus of cp2 was apparently blocked as no sequence was obtained. The 15 amino acids at the $NH_2$-terminus of cp3 were determined to be LQVASLTDIGELSSV, as shown in SEQUENCE I.D. NO. 2 and SEQUENCE I.D. NO. 6. This sequence is an exact match to the derived protein sequence encoded by nucleotides 3144-3188. Likewise, the 15 amino acides at the $NH_2$-terminus of cp1, VSLGRSFENGVLIGS, as shown in SEQUENCE I.D. NO. 5 and SEQUENCE I.D. NO. 7, are an exact match to the derived protein sequence encoded by nucleotides 3750-3794. Both proteins must be derived by proteolytic cleavage of the large polyprotein. The Gln/Leu cleavage at the $NH_2$-terminus of cp3 and Gln/Val cleavage at the $NH_2$-terminus of cp1 are dipeptide cleavage sites that may be used by animal picornavirus 3C proteases, according to Krausslich and Wimmer, Ann. Rev. Biochem., 57, 754 (1988), which could indicate that the 3C-similar region of the MCDV may function in capsid protein processing. Assuming that cp3 begins with the Leu at the Gln/Leu cleavage and ends with the Gln at the Gln/Val cleavage for cp1, cp3 would have a derived MW of 21,933, a little less than the 24.5 kd MW determined by SDS gel electrophoresis. Although protein sequence was not obtained for cp2, the position of clones p7E6 and p7D7, and the finding that protein fusions expressed from the pEX vector for the PstI fragments 2076-2619 and 2613-3149 reacted positively with cp2-specific antiserum (McMullen, unpublished), is consistent with cp2 preceding cp3 in the polyprotein similar to the order of vp2-vp3-vp1 for the animal picornaviruses. However, it is still not known if the coding region for cp2 immediately preceds cp3.

The overall genome structure of MCDV-type strain is shown in Figure 2. MCDV genome organization resembled that of the animal picornaviruses, a single

large polyprotein in which the capsid proteins are encoded 5' of the proteins presumed to be involved in genome replication. Depending on the exact location of cp2, the MCDV genome can encode up to 78 kd of protein 5' of the capsid proteins for which there are no corresponding animal picornavirus protein. This region may encode plant virus specific functions such as cell-to-cell movement or helper protein for insect transmission. Because MCDV is a phloem restricted virus, there is no evidence for a virus-encoded cell-to-cell movement protein. However, there is evidence for the presence of an insect transmission helper component in MCDV-infected plants according to Hunt et al., Phytopathology, 78, 449 (1988). The presence of plant-virus-specific proteins at the $NH^2$-terminus of the polyprotein would allow addition of these proteins without disruption of the cp proteins-replication functions genome structure typical of picornaviruses.

3. Design of the plasmid vector.

The gene MCDV coat protein 3 was placed under control of tandem cauliflower mosaic virus 35S promoters isolated from the 1841 strain of the virus, and a polyadenylation signal sequence obtained from the potato proteinase inhibitor II (Pin II) gene that exhibits enhancer-like activity. The chimeric gene also included a 79 bp sequence Ω' from the 5' leader region of tobacco mosaic virus (TMV) that functions as a translational enhancer; and a Zea mays alcohol dehydrogenase 1, intron 1 fragment (ADH) spanning nucleotides 119-672, trimmed to 557 bp with Bal 31 nuclease, which has been shown to function as an enhancer of gene expression in monocots. The plasmids were grown in E. coli and purified by the known polyethylene glycol precipitation method of Sambrook et al., Molecular Cloning, 1, 40 (1989). Purity was confirmed by electrophoretic analysis of the DNA fragments obtained after digestion with restriction endonucleases. The plasmid was designated pPHI1406 and the sequence is shown in SEQUENCE I.D. No. 1.

4. Preparation of the recipient organism.

Separately, an embryogenic cell suspension line 54-68-5 was established from immature embryos obtained from a cross between a line derived from the public inbred corn line B73 and a WX 1-9 translocation stock of public inbred corn line W23.

5. Transformation

Suspension cells from (4) were bombarded with 1 µl aliquots of a 30 µl mixture containing 10 µg of purified plasmid DNA (5 µg of the MCDV plasmid pPHI1406 (SEQUENCE I.D. No. 1), and 5 µg of the same plasmid in which the BAR

(Basta resistance) gene was substituted for the MCDV cp3 gene) precipitated onto 1 µm tungsten particles as described by numerous articles including Klein, T.M., et al., 1988 (May) Bio/Technology 6:559-563; Klein, T. M., et al., 1988 (June) Proc. Natl. Acad. Sci. USA 85:4305-4309; T. M. Klein, et al., "Stable Genetic Transformation of Intact Applicant Nicotiana Cells by the Particle Bombardment Process", Proc. Natl. Acad. Sci. USA, Vol. 85, November 1988, pp. 8502-8505; D. T. Tomes, et al., "Transgenic Tobacco Plants and their Progeny Derived by Microprojectile Bombardment of Tobacco Leaves", Plant Molecular Biology, Vol. 14, No. 2, February, 1990, pp. 261-268, Kluwer Academic Publishers, BE; and M. C. Ross, et al., "Transient and Stable Transgenic Cells and Calli of Tobacco and Maize Following Microprojectile Bombardment", J. Cell. Biochem., Suppl. 13D, 27th March - April 1989, P. 268, Abstract No. M. 149, Alan R. Liss, Inc. New York, US; and plated onto selective medium containing 5 ppb phosphinothricin (Basta™).

Following a prolonged period of selection and callus growth, regeneration was initiated by placing callus on a Murashige & Skoog medium modified by addition of 0.5 mg/l 2,4-D and 5 ppb Basta. Embryogenic callus was selected and transferred to medium lacking 2,4-D and kept in a lighted growth room. Germinated plantlets were placed in culture tubes and finally planted out into soil in pots in the greenhouse.

More than 150 $R_0$ (recombinant) plants were obtained, representing twenty independent transformation events. Transformation was confirmed by PCR amplification of a DNA fragment spanning part of the MCDV coat protein gene and the CaMV promoter. Genomic DNA samples, in which a fragment of the expected size was successfully amplified were presumed to be transformed. These plants were pollinated with pollen from non-transgenic B73 plants and the resulting $R_1$ seed was planted in a field trial under USDA supervision. The resulting plants exhibited a virus resistant phenotype, i.e., they survived and set seed under virus infection conditions in which non-transgenic plants died prematurely, as seen in the following table:

Field Test Results

| | Transgenic* | Control |
|---|---|---|
| Number of Plants | 379 | 32 |
| Number of Harvestable Ears | 52 | 0 |
| % Harvested vs. Total | 13.7% | 0% |

The screening was performed in a manner to insure maximum infection levels and severity. Thus, the level of resistance seen in this extreme test corresponds to effective, usable virus tolerance when the transformants of this invention are used under normal farming conditions.

The MCDV resistance is a simply inherited, dominant trait and can, if desired, be introduced into other maize varieties by simple crossing or backcrossing. In addition to providing resistance to MCDV, this invention is also capable of conferring resistance to viruses to which plants obtain cross-resistance through infection by MCDV. In the field test described above, resistance to maize dwarf mosaic virus strain A (MDMV-A) was also observed. Accordingly, this invention provides resistance to that virus as well.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: McMullen, Michael D.; Roth, Bradley A.; Townsend, Rod

(ii) TITLE OF INVENTION: MAIZE CHLOROTIC DWARF VIRUS RESISTANCE

(iii) NUMBER OF SEQUENCES: 7

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Pioneer Hi-Bred International, Inc.

(B) STREET: 700 Capital Square, 400 Locust Street

(C) CITY: Des Moines

(D) STATE: Iowa

(E) COUNTRY: United States

(F) ZIP: 50309

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette, 3.5 inch, 1.44 Mb storage

(B) COMPUTER: IBM Compatible

(C) OPERATING SYSTEM: MS-DOS, Microsoft Windows

(D) SOFTWARE: Microsoft Windows Notepad

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Roth, Michael J.

(B) REGISTRATION NUMBER: 29,342

(C) REFERENCE/DOCKET NUMBER: 0235 US

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (515) 245-3594

(B) TELEFAX: (515) 245-3634

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5033 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA
(A) DESCRIPTION: transformation plasmid pPHI1406
(iii) HYPOTHETICAL: No
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG 50
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CGGCATCAGA GCAGATTGTA CTGAGAGTGC ACCATATGCG GTGTGAAATA 200
CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGGCGCC ATTCGCCATT 250
CAGGCTGCGC AACTGTTGGG AAGGGCGATC GGTGCGGGCC TCTTCGCTAT 300
TACGCCAGCT GGCGAAAGGG GGATGTGCTG CAAGGCGATT AAGTTGGGTA 350
ACGCCAGGGT TTTCCCAGTC ACGACGTTGT AAAACGACGG CCAGTGCCAA 400
GCTCAGATCT GAGCTTCTAG AAATCCGTCA ACATGGTGGA GCACGACACT 450
CTCGTCTACT CCAAGAATAT CAAAGATACA GTCTCAGAAG ACCAAAGGGC 500
TATTGAGACT TTTCAACAAA GGGTAATATC GGGAAACCTC CTCGGATTCC 550
ATTGCCCAGC TATCTGTCAC TTCATCAAAA GGACAGTAGA AAAGGAAGGT 600
GGCACCTACA AATGCCATCA TTGCGATAAA GGAAAGGCTA TCGTTCAAGA 650
TGCCTCTGCC GACAGTGGTC CCAAAGATGG ACCCCCACCC ACGAGGAGCA 700
TCGTGGAAAA AGAAGACGTT CCAACCACGT CTTCAAAGCA AGTGGATTGA 750
TGTGATGCTC TAGAAATCCG TCAACATGGT GGAGCACGAC ACTCTCGTCT 800
ACTCCAAGAA TATCAAAGAT ACAGTCTCAG AAGACCAAAG GGCTATTGAG 850
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TCTCCACTGA CGTAAGGGAT GACGCACAAT CCCACTATCC TTCGCAAGAC 1150
CCTTCCTCTA TATAAGGAAG TTCATTTCAT TTGGAGAGGA CGAGCTGCAG 1200
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ACATCGTCCA GCACTGCTAT TACCGTGTGG TCCATCCGAC AGTCTGGCTG 1550
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CAATGATACC GCGAGACCCA CGCTCACCGG CTCCAGATTT ATCAGCAATA 4150
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AATAAGGGCG ACACGGAAAT GTTGAATACT CATACTCTTC CTTTTTCAAT 4850

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GAATGTATTT AGAAAAATAA ACAAATAGGG GTTCCGCGCA CATTTCCCCG 4950
AAAAGTGCCA CCTGACGTCT AAGAAACCAT TATTATCATG ACATTAACCT 5000
ATAAAAATAG GCGTATCACG AGGCCCTTTC GTC 5033

(2) INFORMATION FOR SEQ ID NO: 2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 bases
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: viral RNA
(A) DESCRIPTION: RNA codons for first 15 amino acids at 5' end of MCDV coat protein 3 (CP3)
(iii) HYPOTHETICAL: No
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:
CUG CAG GUG GCA UCU CUU ACA GAC AUA GGA GAA UUG AGC AGU GUG 45
Leu Gln Val Ala Ser Leu Thr Asp Ile Gly Asp Leu Ser Ser Val
(2) INFORMATION FOR SEQ ID NO: 3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 bases
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: synthetic DNA
(A) DESCRIPTION: sequencing primer
(iii) HYPOTHETICAL: No
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:
GGTCTACTCA CGGCACGCCA 20
(2) INFORMATION FOR SEQ ID NO: 4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11785 bases
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: viral RNA

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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GCCUGGAUUU GCUAUUAGAA UGUCCCUAGC CGGUGAUAAC CUUGAGUCCC         350
CGUCAUAGGA CUACUUUUGU UUGCUUAGUA AUACAUUGGG ACCACCCGCA         400
UGGAGCUCUG AGCCUACCAU ACAUAGUACA UUUUCCGAGG GAUUGUCUUU         450
UGAUA       AUG AUG CAG ACA AAC AAC AAC CAA AAU CCC             485
            Met Met Gln Thr Asn Asn Asn Gln Asn Pro
ACU CAA GGA AGC AUU CCU GAG AAC UCC UCA CAA GAU CGC AAC UUA 530
Thr Gln Gly Ser Ile Pro Glu Asn Ser Ser Gln Asp Arg Asn Leu
GGA GUG CCC GCU GGA UAU UCU UUA AGC GUU GAG GAC CCC UUC GGG 575
Gly Val Pro Ala Gly Tyr Ser Leu Ser Val Glu Asp Pro Phe Gly
AAC CGG UCU GAC UUU CAU AUC CCA GUG CAC CAA AUC AUU CGG GAA 620
Asn Arg Ser Asp Phe His Ile Pro Val His Gln Ile Ile Arg Glu
GAG AUU GAU CGU CCA AAU UGG GUU CCU AUA UGU UCA AAC GAU UUU 665
Glu Ile Asp Arg Pro Asn Trp Val Pro Ile Cys Ser Asn Asp Phe
CAU CUU AAC AGU GAG GAU UAU UGU GAG GAG UGC GAA UCU GAA CGG 710
His Leu Asn Ser Glu Asp Tyr Cys Glu Glu Cys Asp Ser Asp Arg
AUC AAA AAU UUC GAA AUA UUC AGA UCA CAG AAU UUG AUU GAC CAA 755
Ile Lys Asn Phe Asp Ile Phe Arg Ser Gln Asn Leu Ile Asp Gln
CAC CUA AAU CUC UGU ACU GAU UCA AAG GAU UGU GAU CAU UUU UCU 800
His Leu Asn Leu Cys Thr Asp Ser Lys Asp Cys Asp His Phe Ser
UGU UUU UCC ACG AGU ACA AGU UGC AGA UUU UGC CCU UUU UGC UUA 845
Cys Phe Ser Thr Ser Thr Ser Cys Arg Phe Cys Pro Phe Cys Leu
UUC AUU UUU AAU UUG GAU AAA UUU UAC AAA CAA AAU CUA UAU UUG 890
Phe Ile Phe Asn Leu Asp Lys Phe Tyr Lys Gln Asn Leu Tyr Leu
AUU AGU CGU CAG GCU CUA GCU AGA UUG UUC CAC GGA AGC GCC GAA 935
Ile Ser Arg Gln Ala Leu Ala Arg Leu Phe His Gly Ser Ala Asp
GAG UUA CUC AGU AGA GCG AUU UUC UUU ACG UAU AAU AUU UGU AUU 980
Glu Leu Leu Ser Arg Ala Ile Phe Phe Thr Tyr Asn Ile Cys Ile
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AAG UUG UUU CAU CCA GAC CUU AGG CCU AGU AUU ACG UCU CCC CCU 1070
Lys Leu Phe His Pro Asp Leu Arg Pro Ser Ile Thr Ser Pro Pro
UAU GCU AGU GAU UGG GUU AUG UGU GAU AAU GCU AAA CAU CUU UUU 1115
Tyr Ala Ser Asp Trp Val Met Cys Asp Asn Ala Lys His Leu Phe
GAG UGU CUU GGC CUU GGU GAC ACG ACC AGA GGA CAC CUA UAU GGA 1160
Glu Cys Leu Gly Leu Gly Asp Thr Thr Arg Gly His Leu Tyr Gly
CUU AUU AGC GAG AAU GCA UAU UGG AAC GCC ACG UGC UCA AAA UGC 1205
Leu Ile Ser Glu Asn Ala Tyr Trp Asn Ala Thr Cys Ser Lys Cys
GGA GCC UGU UGU CAG GGA GCA AAU GCC CGU ACG GCG AUA CCG AUA 1250
Gly Ala Cys Cys Gln Gly Ala Asn Ala Arg Thr Ala Ile Pro Ile
GUG AUG GCG UUG CAG UAC UGC AGG GUG GAU GUG UAU UAU AGU GAG 1295
Val Met Ala Leu Gln Tyr Cys Arg Val Asp Val Tyr Tyr Ser Glu
UAC UAU UUA UAC CAC AUC UAC GCU CCG GAA GAG AGA AUG AAG AUU 1340
Tyr Tyr Leu Tyr His Ile Tyr Ala Pro Asp Glu Arg Met Lys Ile
GAU CAA CAG ACA GCA CAC UUG CUA CAC AGU AUA AUC CGA GGA GCA 1385
Asp Gln Gln Thr Ala His Leu Leu His Ser Ile Ile Arg Gly Ala
CCA GCA GUG GAU UGC UCU GAG UUA UCU CAG GAG CCA AUU CAC AGG 1430
Pro Ala Val Asp Cys Ser Glu Leu Ser Gln Glu Pro Ile His Arg
AUG GUA AUG GAU AGC UCA AAG UUA GUG GCA CUG GAU UCG ACA AUC 1475
Met Val Met Asp Ser Ser Lys Leu Val Ala Leu Asp Ser Thr Ile
AGG CAU CCU AAG AGC CAA GGA AGU UUG CUC GAU UCA GAA UGC GAU 1520
Arg His Pro Lys Ser Gln Gly Ser Leu Leu Asp Ser Asp Cys Asp
CAU GAG UUU AUU CUA AGA ACG UCC CAU GGU AUC AAA AUA CCG AUG 1565
His Glu Phe Ile Leu Arg Thr Ser His Gly Ile Lys Ile Pro Met
AGU AAG UCU UUA UUU AUA UCA UUU CUU ACC AUG GGA GCU UAU CAU 1610
Ser Lys Ser Leu Phe Ile Ser Phe Leu Thr Met Gly Ala Tyr His
GGG UAU GCU CAU GAU GAU CAG CAG GAG CAA AAU GCG AUA AUA UCU 1655
Gly Tyr Ala His Asp Asp Gln Gln Glu Gln Asn Ala Ile Ile Ser
UUU GGU GGG AUG CCC GGA GUC AAU UUG GCU UGU AAC AAA AAU UUC 1700
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CUG AGA AUG CAU AAG UUG UUU UAU UCU GGA AGU UUU AGG CGC AGA 1745
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CCC CUG UUU AUG AGC CAA AUU CCC UCU ACG AAU GCC ACC GCU CAG 1790
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Ser Gly Phe Asn Asp Asp Asp Phe Asp Arg Leu Met Ala Asp Glu
GGU GUG CAU GUC AAA GUC GAG CGU CCA AUA GCA GAG AGG UUU GAU 1880
Gly Val His Val Lys Val Glu Arg Pro Ile Ala Glu Arg Phe Asp
UAU GAG GAC GUU AUU GAU AUU UAC GAU GAG ACC GAC CAC GAC AGG 1925
Tyr Glu Asp Val Ile Asp Ile Tyr Asp Glu Thr Asp His Asp Arg
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Thr Arg Ala Leu Gly Leu Gly Gln Val Phe Gly Gly Leu Leu Lys
GGA AUU UCU CAU UGU GUA GAU AGC CUA CAU AAG GUA UUU GAU UUC 2015
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ACA AAA GAA GCU UUU GAA UUA AUA CGA UCA AGU AUA AAG AAG CUU 2195
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UCC GAG GGC AUU GAC AAA AUC ACG AAG AUG AAU GCU ACG AAC UUU 2240
Ser Glu Gly Ile Asp Lys Ile Thr Lys Met Asn Ala Thr Asn Phe
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Asp Arg Ile Leu Asp Gly Ile Lys Pro Ile Glu Ser Arg Leu Thr
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GCU CUG GUC CAG GCC GUG AAA GAC UUG AAA AUC AUG AAA GAG GCG 2375
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Lys Val Ser Asp Gly Gln Asp Lys Val Leu Ala Asp Leu Asp Leu
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Trp Asp Ala Asn Gly Cys Ala Thr Arg Gln Gly Ile Ala Thr Thr
GUU CAG CUG ACU GGU UUG CCC AAA ACA UUU AUU GAA GCU CAC AGC 2915
Val Gln Leu Thr Gly Leu Pro Lys Thr Phe Ile Asp Ala His Ser
UCA UCA GAA ACG AUA AUC GUG GUA AAG AAU UCC AAU AUA CAA UCC 2960
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GCG AUU UGU CUA AGU GGA AGU GAG CAC UCG UUU GGG AGA AUG GGA 3005
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Ile Leu Lys Ile Cys Cys Leu Asn Thr Leu Asn Ala Pro Lys Asp
GCU ACA CAG CAA GUG GCU GUG AAC GUC UGG AUU AAG UUU GAC GGA 3095
Ala Thr Gln Gln Val Ala Val Asn Val Trp Ile Lys Phe Asp Gly
GUU AAA UUU CAC GUU UAU UCU UUA AGG AAA AAU CCA GUC GUU UCG 3140
Val Lys Phe His Val Tyr Ser Leu Arg Lys Asn Pro Val Val Ser
CAA CUG CAG GUG GCA UCU CUU ACA GAC AUA GGA GAA UUG AGC AGU 3185
Gln Leu Gln Val Ala Ser Leu Thr Asp Ile Gly Asp Leu Ser Ser
GUG GUU GCU ACU GGU UCU UGG UCU ACU ACC UCG GCU ACU AAU UUG 3230
Val Val Ala Thr Gly Ser Trp Ser Thr Thr Ser Ala Thr Asn Leu
AUG GAA UUA AAC AUU CAU CCC ACC UCC UGU GCU AUU CAG AAC GGA 3275
Met Asp Leu Asn Ile His Pro Thr Ser Cys Ala Ile Gln Asn Gly
UUG AUA ACA CAG ACA CCA UUG AGU GUU UUA GCU CAU GCU UUU GCA 3320
Leu Ile Thr Gln Thr Pro Leu Ser Val Leu Ala His Ala Phe Ala
AGG UGG AGA GGA UCG UUG AAA AUU UCC AUC AUU UUC GGA GCG AGU 3365
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UUG UUU ACC CGA GGA CGA AUC UUA GCC GCU GCU GUG CCC GUU GCU 3410
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AAG CGC AAA GGU ACC AUG AGC CUU GAC GAG AUU AGU GGG UAU CAU 3455
Lys Arg Lys Gly Thr Met Ser Leu Asp Glu Ile Ser Gly Tyr His
AAU GUU UGC UGC UUA UUG AAU GGU CAG CAA ACU ACA UUU GAA UUG 3500
Asn Val Cys Cys Leu Leu Asn Gly Gln Gln Thr Thr Phe Asp Leu
GAA AUC CCA UAU UAU UCU GUG GGC CAA GAU UCU UUC GUG UAC CGU 3545
Asp Ile Pro Tyr Tyr Ser Val Gly Gln Asp Ser Phe Val Tyr Arg
GAU GCU CUU UUU GAU AUC UCU GCG CAC GAU GGG AAU UUU AUG AUU 3590
Asp Ala Leu Phe Asp Ile Ser Ala His Asp Gly Asn Phe Met Ile
ACU CGC UUG CAU CUC GUG AUA CUG GAU AAA UUG GUA AUG AGC GCU 3635
Thr Arg Leu His Leu Val Ile Leu Asp Lys Leu Val Met Ser Ala
AAU GCG AGC AAC AGC AUA AAU UUU UCC GUG ACU CUU GGA CCA GGU 3680
Asn Ala Ser Asn Ser Ile Asn Phe Ser Val Thr Leu Gly Pro Gly
UCU GAU UUG GAA UUG AAA UAU CUU GCA GGA GUA CAU GGG CAG CGC 3725
Ser Asp Leu Asp Leu Lys Tyr Leu Ala Gly Val His Gly Gln Arg
AUA GUC CGC GAG UUG AAG AUG CAG GUU UCA UUG GGU CGG UCA UUU 3770
Ile Val Arg Glu Leu Lys Met Gln Val Ser Leu Gly Arg Ser Phe
GAG AAU GGA GUG CUU AUU GGU AGU GGC UUC GAC GAC UUG CUA CAA 3815
Glu Asn Gly Val Leu Ile Gly Ser Gly Phe Asp Asp Leu Leu Gln
AGA UGG AGU CAU UUG GUG UCC AUG CCU UUU AAU GCA AAA GGA GAC 3860
Arg Trp Ser His Leu Val Ser Met Pro Phe Asn Ala Lys Gly Asp
AGC GAU GAG AUC CAA GUC UUU GGC UAU AUC AUG ACU GUU GCC CCG 3905
Ser Asp Glu Ile Gln Val Phe Gly Tyr Ile Met Thr Val Ala Pro
GCG UAU CGU UCC CUU CCA GUC CAC UGC ACG CUG CUA AGU UGG UUU 3950
Ala Tyr Arg Ser Leu Pro Val His Cys Thr Leu Leu Ser Trp Phe
UCA CAA UUA UUC GUG CAG UGG AAA GGU GGU AUA AAG UAU AGA CUA 3995
Ser Gln Leu Phe Val Gln Trp Lys Gly Gly Ile Lys Tyr Arg Leu
CAC AUU GAU UCA GAA GAG CGC AGA UGG GGU GGA UUC AUC AAA GUU 4040
His Ile Asp Ser Asp Glu Arg Arg Trp Gly Gly Phe Ile Lys Val
UGG CAU GAC CCA AAU GGC UCU UUG GAU GAA GGG AAA GAA UUU GCU 4085
Trp His Asp Pro Asn Gly Ser Leu Asp Asp Gly Lys Asp Phe Ala
AAA GCG GAU AUU CUA UCG CCA CCA GCC GGA GCU AUG GUU CGU UAU 4130
Lys Ala Asp Ile Leu Ser Pro Pro Ala Gly Ala Met Val Arg Tyr
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UGG AAC UAU UUA AAU GGA GAC UUG GAG UUU ACA GUA CCA UUU UGU 4175
Trp Asn Tyr Leu Asn Gly Asp Leu Glu Phe Thr Val Pro Phe Cys
GCU AGA ACC AGU ACG CUG UUC AUA CCA AAA GCU AUG AUU GCC ACC 4220
Ala Arg Thr Ser Thr Leu Phe Ile Pro Lys Ala Met Ile Ala Thr
GAU UCA AAG UCA UGG AUU CUG AAC UAC AAC GGU ACA UUG AAU UUC 4265
Asp Ser Lys Ser Trp Ile Leu Asn Tyr Asn Gly Thr Leu Asn Phe
GCG UAC CAA GGA GUA GAU GAC UUC ACA AUU ACA GUG GAA ACA AGU 4310
Ala Tyr Gln Gly Val Asp Asp Phe Thr Ile Thr Val Asp Thr Ser
GCA GCC GAC GAC UUU GAA UUU CAC GUU CGA ACA GUU GCA CCC CGC 4355
Ala Ala Asp Asp Phe Asp Phe His Val Arg Thr Val Ala Pro Arg
GCU GGA AAG GUC AAC GAA GCU UUU GCC AAA UUG GAG UAC GCU UCU 4400
Ala Gly Lys Val Asn Asp Ala Phe Ala Lys Leu Glu Tyr Ala Ser
GAU UUA AAG GAU AUC AAA GAA UCU CUG ACA UCU UCC ACU CGU UUG 4445
Asp Leu Lys Asp Ile Lys Asp Ser Leu Thr Ser Ser Thr Arg Leu
AAA GGG CCU CAU UAU AAA ACG AAA AUU ACC UCA AUA GAG CCA AAU 4490
Lys Gly Pro His Tyr Lys Thr Lys Ile Thr Ser Ile Glu Pro Asn
AAA AUU GAU GAA AAU GAG UCC UCA CGU GGU AAA GAU AAC AAG UCA 4535
Lys Ile Asp Asp Asn Glu Ser Ser Arg Gly Lys Asp Asn Lys Ser
AAU UCG AAA UUU GAG GAC UUA CUC AAU GCA ACA GCU CAG AUG GAU 4580
Asn Ser Lys Phe Glu Asp Leu Leu Asn Ala Thr Ala Gln Met Asp
UUU GAU CGA GCC ACA GCG AAC GUU GGG UGU GUG CCA UUC UCC AUU 4625
Phe Asp Arg Ala Thr Ala Asn Val Gly Cys Val Pro Phe Ser Ile
GCA AAG ACA GCA AAG GUG CUU UCG GAA CGC GAG ACG UGU AAG AAG 4670
Ala Lys Thr Ala Lys Val Leu Ser Asp Arg Glu Thr Cys Lys Lys
AUG GCA GAU GUG UUA GAU UUC ACA CAC UCA UGU UUG AAC UUA GAC 4715
Met Ala Asp Val Leu Asp Phe Thr His Ser Cys Leu Asn Leu Asp
AGU CAA CCU GCG GCG GCA AGA UUA GCA GCG GCC AUU UCU CAA AUA 4760
Ser Gln Pro Ala Ala Ala Arg Leu Ala Ala Ala Ile Ser Gln Ile
GCA CCU AUU AUG GAG AGC AUC GGU AGA ACC ACU CAA AGC GUA GAG 4805
Ala Pro Ile Met Glu Ser Ile Gly Arg Thr Thr Gln Ser Val Glu
GAA AAA UUG GCU UCU GUG GAU ACA UUU AGG GAC AAA AUC AUG GCU 4850
Asp Lys Leu Ala Ser Val Asp Thr Phe Arg Asp Lys Ile Met Ala
CUA AUU UCA AAC GUG CUU GGG GAU ACU CUA CCU GGA CUG GCC-AUU 4895
Leu Ile Ser Asn Val Leu Gly Asp Thr Leu Pro Gly Leu Ala Ile
GCU GAC UUC AAA AAA GGA AAA UAU GUG UGG GCC UCG UUC CUG ACA 4940
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Ala Asp Phe Lys Lys Gly Lys Tyr Val Trp Ala Ser Phe Leu Thr
AUG AUA GCC GCU UGC GUA GUA GCU UGG GCU GCC ACU AGC AAG AAA 4985
Met Ile Ala Ala Cys Val Val Ala Trp Ala Ala Thr Ser Lys Lys
AGC UUC UUG AAA AGA UUU GCA GUG GUA GCU AUG AUA AUU UGG AGC 5030
Ser Phe Leu Lys Arg Phe Ala Val Val Ala Met Ile Ile Trp Ser
CCA UUU CUC GCA AGU AAA AUA UGG GCG CUU GGU ACA UGG AUU AGG 5075
Pro Phe Leu Ala Ser Lys Ile Trp Ala Leu Gly Thr Trp Ile Arg
AAG AGC UGG AGU AAG CUU UGG CCU AAG UCA GAC UCA UGC CGA CAA 5120
Lys Ser Trp Ser Lys Leu Trp Pro Lys Ser Asp Ser Cys Arg Gln
CAC UCU UUG GCA GGC CUG UGU GAA AGU GUG UUC ACA UCA UUC AAG 5165
His Ser Leu Ala Gly Leu Cys Asp Ser Val Phe Thr Ser Phe Lys
GAU UUC CCU GAC UGG UUU AAA UCA GGA GGA AUC ACG AUU GUG ACG 5210
Asp Phe Pro Asp Trp Phe Lys Ser Gly Gly Ile Thr Ile Val Thr
CAA GUU UGC ACA GUA UUA CUG ACG AUA GUG AGU CUG AUU ACA CUU 5255
Gln Val Cys Thr Val Leu Leu Thr Ile Val Ser Leu Ile Thr Leu
GGA ACU AUA CCA AGC ACG AAA CAA AAU GCU ACG UUC GCA GAC AAA 5300
Gly Thr Ile Pro Ser Thr Lys Gln Asn Ala Thr Phe Ala Asp Lys
UUU AAA GAA UUU GGU AAC AUG AGC AGA GCU ACA ACG UCA AUA GCU 5345
Phe Lys Asp Phe Gly Asn Met Ser Arg Ala Thr Thr Ser Ile Ala
GCA GGU UAC AAG ACG AUA UCA GAG CUG UGU UCG AAA UUC ACC AAU 5390
Ala Gly Tyr Lys Thr Ile Ser Glu Leu Cys Ser Lys Phe Thr Asn
UAC UUG GCU GUA ACC UUC UUU GGG GCG CAA GUU GAU GAC GAU GCU 5435
Tyr Leu Ala Val Thr Phe Phe Gly Ala Gln Val Asp Asp Asp Ala
UUC AAG GGU UUG GUA GCG UUC AAC GUU AAG GAA UGG AUU CUU GAA 5480
Phe Lys Gly Leu Val Ala Phe Asn Val Lys Asp Trp Ile Leu Asp
GUG AAA AAC CUG UCU CUU GAG GAA AAC AAA UUU AGU GGU UUU GGU 5525
Val Lys Asn Leu Ser Leu Glu Asp Asn Lys Phe Ser Gly Phe Gly
GGU GAU GAG CAU CUU GUC AAG GUU AGA CAU UUA UAU GAU AAA UCU 5570
Gly Asp Glu His Leu Val Lys Val Arg His Leu Tyr Asp Lys Ser
GUG GAA AUA ACC UAU AAG UUG CUC CAG AAA AAU CGA GUU CCC AUU 5615
Val Asp Ile Thr Tyr Lys Leu Leu Gln Lys Asn Arg Val Pro Ile
GCU AUG CUU CCU AUC AUC CGA GAC ACG UGU AAG AAG UGC GAG GAU 5660
Ala Met Leu Pro Ile Ile Arg Asp Thr Cys Lys Lys Cys Glu Asp
UUG CUA AAC GAG AGU UAU ACU UAC AAA GGU AUG AAA ACU CCG CGC 5705
Leu Leu Asn Glu Ser Tyr Thr Tyr Lys Gly Met Lys Thr Pro Arg
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GUG GAC CCA UUC UAU AUA UGC CUU UUU GGA GCA CCU GGA GUU GGC 5750
Val Asp Pro Phe Tyr Ile Cys Leu Phe Gly Ala Pro Gly Val Gly
AAG UCC ACA GUG GCA UCG AUG AUU GUU GAC GAU UUG UUG GAU GCU 5795
Lys Ser Thr Val Ala Ser Met Ile Val Asp Asp Leu Leu Asp Ala
AUG GGC GAA CCU AAG GUU GAU AGG AUC UAU ACG CGA UGC UGU UCU 5840
Met Gly Asp Pro Lys Val Asp Arg Ile Tyr Thr Arg Cys Cys Ser
GAU CAA UAU UGG AGC AAU UAU CAC CAC GAG CCA GUU AUU UGU UAU 5885
Asp Gln Tyr Trp Ser Asn Tyr His His Glu Pro Val Ile Cys Tyr
GAC GAC UUG GGG GCA AUC AGC AGA CCA GCG AGU UUA UCA GAC UAU 5930
Asp Asp Leu Gly Ala Ile Ser Arg Pro Ala Ser Leu Ser Asp Tyr
GGG GAG AUA AUG GGA AUC AAA UCG AAC AGA CCA UAC UCC CUA CCU 5975
Gly Glu Ile Met Gly Ile Lys Ser Asn Arg Pro Tyr Ser Leu Pro
AUG GCU GCU GUU GAU GAG AAA GGA AGG CAU UGU UUA UCG CGA UAC 6020
Met Ala Ala Val Asp Glu Lys Gly Arg His Cys Leu Ser Arg Tyr
CUC AUU GCU UGU ACA AAU UUA ACC CAU CUG GAC GAU ACG GGC GAU 6065
Leu Ile Ala Cys Thr Asn Leu Thr His Leu Asp Asp Thr Gly Asp
GUG AAA ACA AAG GAU GCC UAC UAU CGC AGA AUC AAU GUC CCA GUG 6110
Val Lys Thr Lys Asp Ala Tyr Tyr Arg Arg Ile Asn Val Pro Val
ACA GUG ACG AGA GAA GUA ACC GCC AUG AUG AAC CCC GAG GAC CCA 6155
Thr Val Thr Arg Asp Val Thr Ala Met Met Asn Pro Glu Asp Pro
ACU GAU GGA CUA CGU UUC ACC GUG GAG CAA GUG CUU GAU GGA GGU 6200
Thr Asp Gly Leu Arg Phe Thr Val Glu Gln Val Leu Asp Gly Gly
AGA UGG AUU AAU GUU ACU GAA AGC CGU CUC CUC AAU GGA AGG AUG 6245
Arg Trp Ile Asn Val Thr Asp Ser Arg Leu Leu Asn Gly Arg Met
CCA UUC AGG GCU GAA GAU CUC AUG AAC AUG AAC UAC AGU UAC UUU 6290
Pro Phe Arg Ala Asp Asp Leu Met Asn Met Asn Tyr Ser Tyr Phe
AUG GAG UUU CUC AAG AUG UAU GCU GCU UUA UAU AUG GAA AAU CAA 6335
Met Glu Phe Leu Lys Met Tyr Ala Ala Leu Tyr Met Asp Asn Gln
AAC AUG UUG GUG GCA AAA UUG AGA GGA ACA GAG AUC CCA GAA UCA 6380
Asn Met Leu Val Ala Lys Leu Arg Gly Thr Glu Ile Pro Asp Ser
CGU AGU UCA GAG AAU GAA GAA CUU GAA UUC GAU UAU UUG GCU ACA 6425
Arg Ser Ser Glu Asn Asp Asp Leu Asp Phe Asp Tyr Leu Ala Thr
GCU CAG AUG GAC CAU ACA GUG ACA UUU GGG GAA CUA GUU ACC AAA 6470
Ala Gln Met Asp His Thr Val Thr Phe Gly Asp Leu Val Thr Lys
UUC AAC UCG UAU AAG CUU ACU GGG AAA CAA UGG AAC AAG AGG CUC 6515
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Phe Asn Ser Tyr Lys Leu Thr Gly Lys Gln Trp Asn Lys Arg Leu
UGU GAA CUU GGA UGG ACA UCU CUA GAC GGA UGG AAC ACG AAC AAG 6560
Cys Asp Leu Gly Trp Thr Ser Leu Asp Gly Trp Asn Thr Asn Lys
AUU AUG AGA UUC GAC GAU CUA GUU GCC GGA UUC UGU GGU UGC UCA 6605
Ile Met Arg Phe Asp Asp Leu Val Ala Gly Phe Cys Gly Cys Ser
AGG AAU GAG AAU UGC AAU UUU GAC UUC UAU CAU CAG AGA CUU CAA 6650
Arg Asn Glu Asn Cys Asn Phe Asp Phe Tyr His Gln Arg Leu Gln
GCA UGU UUG AAC AAG AAA GGG UUU GCU CCC GCA UAU CAA UAU UUC 6695
Ala Cys Leu Asn Lys Lys Gly Phe Ala Pro Ala Tyr Gln Tyr Phe
AAC CUU CAC AAG UUG AAU UCA GAC ACC CAG AAG ACA GAG CUC AAG 6740
Asn Leu His Lys Leu Asn Ser Asp Thr Gln Lys Thr Glu Leu Lys
CUU AAA UGC GGG ACA ACU GCU GAA GAU UUA UUC AGA CAA GCU GAC 6785
Leu Lys Cys Gly Thr Thr Ala Asp Asp Leu Phe Arg Gln Ala Asp
UUG AUG GUC AUA UUC UCC UAC CUC UUA UUU GUU GCG AGA AUU GGG 6830
Leu Met Val Ile Phe Ser Tyr Leu Leu Phe Val Ala Arg Ile Gly
GUG AGU GGA UCU CAU GUG UGU CUG UCA UAU AAC AUG UUG AAC GUC 6875
Val Ser Gly Ser His Val Cys Leu Ser Tyr Asn Met Leu Asn Val
AAG GAU GUC AAG GAU UUU GAG AUA UGC AGG GAG AAC GUU CUU GAU 6920
Lys Asp Val Lys Asp Phe Glu Ile Cys Arg Glu Asn Val Leu Asp
UUG UCC AGA AAA ACU ACA AUC GAC GGU GAA GAA UGC UAU AUC UGG 6965
Leu Ser Arg Lys Thr Thr Ile Asp Gly Asp Asp Cys Tyr Ile Trp
AAU UUU AUU UCU GAU AUC UUC CCA CGC AUU GUG GCU AAG UAC AAC 7010
Asn Phe Ile Ser Asp Ile Phe Pro Arg Ile Val Ala Lys Tyr Asn
UGU GUU GUG CUU AAC GAC GGA GAG AAG AGA UAC AUC UUC GUG ACU 7055
Cys Val Val Leu Asn Asp Gly Glu Lys Arg Tyr Ile Phe Val Thr
GAC AGC GCG CCC ACU AGG AUC UUU CCC GAU UUG GCU UGG UCA GAU 7100
Asp Ser Ala Pro Thr Arg Ile Phe Pro Asp Leu Ala Trp Ser Asp
CUU AUU UCC GGC AAG CAA GUU GUG AGU CCA AAC AUU AUC AAA GUG 7145
Leu Ile Ser Gly Lys Gln Val Val Ser Pro Asn Ile Ile Lys Val
GCU GGA GAA ACC AAG UCG AAA ACC AUU GCC CCU CUG CUA GCA GAU 7190
Ala Gly Asp Thr Lys Ser Lys Thr Ile Ala Pro Leu Leu Ala Asp
UCC UAC AAG GUU UUC AAG GAU CCG AAG GCA UGG CUU GAG AGG AAC 7235
Ser Tyr Lys Val Phe Lys Asp Pro Lys Ala Trp Leu Glu Arg Asn
AAA GAA UUG AAA GCA GCU CUA GAA ACA GAA GAA UAU AUC GCU CUC 7280
Lys Asp Leu Lys Ala Ala Leu Asp Thr Asp Asp Tyr Ile Ala Leu
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CUC UUU GCU GUU GCA UGU GAA GCU GGU AGA UUC ACU CAA AUU UUA 7325
Leu Phe Ala Val Ala Cys Asp Ala Gly Arg Phe Thr Gln Ile Leu
GAC AAA CCU CCC AGU AGA CGC AAG AUU UUA AAU AUG UCC GAA AGG 7370
Asp Lys Pro Pro Ser Arg Arg Lys Ile Leu Asn Met Ser Asp Arg
UAU AAU GCA UAU AUU GAA CAG GAA AAA GGG CUG AUU GGG AGA CUU 7415
Tyr Asn Ala Tyr Ile Asp Gln Asp Lys Gly Leu Ile Gly Arg Leu
UCU AAA CCA GCA AAG AUA UGC UUA GCC AUA GGA ACU GGA GUU GCG 7460
Ser Lys Pro Ala Lys Ile Cys Leu Ala Ile Gly Thr Gly Val Ala
AUC UUU GGG GCC CUA GCA GGC AUU GGA GUG GGU UUG UUU AAG CUG 7505
Ile Phe Gly Ala Leu Ala Gly Ile Gly Val Gly Leu Phe Lys Leu
AUA GCU CAC UUC AAC AAA GAU GAA GAA GAG GUA GAC GAA AUU GAA 7550
Ile Ala His Phe Asn Lys Asp Asp Asp Glu Val Asp Asp Ile Asp
UUU GAU AUA CUC UCC CCA GAG AUG AGC GGU UCG CAC GAA UCC GGC 7595
Phe Asp Ile Leu Ser Pro Glu Met Ser Gly Ser His Asp Ser Gly
CAA CAU ACC ACG AGG UAC GUC ACG AAG GAG CGA GUU CCA UCC AAA 7640
Gln His Thr Thr Arg Tyr Val Thr Lys Glu Arg Val Pro Ser Lys
CCA GCA AGG AGG CAA CAU GAA UUU GAU CUA AUG UUC GAU AAU CUA 7685
Pro Ala Arg Arg Gln His Asp Phe Asp Leu Met Phe Asp Asn Leu
CCC ACU CCA CAA GUU GAA GAG CUA AAG AGU GAG AUG ACC UGC GCC 7730
Pro Thr Pro Gln Val Asp Glu Leu Lys Ser Glu Met Thr Cys Ala
AGU GCC AGU GAU GAG CAU AAG ACU CAG UAU GUU AAA AGA AGA GUG 7775
Ser Ala Ser Asp Glu His Lys Thr Gln Tyr Val Lys Arg Arg Val
GGA CCU GUA AGC AAA CGU AAG GAU GCU UCG GUA GCA GAA AUU AGU 7820
Gly Pro Val Ser Lys Arg Lys Asp Ala Ser Val Ala Asp Ile Ser
GGA GCU CAU GCG AGU GAU CAG CAU CAU ACA GAA UAC UUG AAA GCA 7865
Gly Ala His Ala Ser Asp Gln His His Thr Asp Tyr Leu Lys Ala
CGC GUU CCA CUC AUG AAA AGA AUA GCU ACC AAA GAG AGC UAU GUU 7910
Arg Val Pro Leu Met Lys Arg Ile Ala Thr Lys Glu Ser Tyr Val
GUA ACU UAC GAU GAC GAA CCC AGC UCU CAU AUU UCC CUA GUU CGC 7955
Val Thr Tyr Asp Asp Asp Pro Ser Ser His Ile Ser Leu Val Arg
AGG AUC CGA CGU ACA CGA CUG GCA AGA GCC AUC AAG CAA AUG GCA 8000
Arg Ile Arg Arg Thr Arg Leu Ala Arg Ala Ile Lys Gln Met Ala
GUC CUG GAG GAC UUC CCA UCU ACC UUG GAA GAG AUA CGA CUU UGG 8045
Val Leu Glu Asp Phe Pro Ser Thr Leu Asp Glu Ile Arg Leu Trp
AGA CAA AAC GCU GCA AAU AAA GGG GUU AUU GUU CCG AAG UAC UCA 8090
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Arg Gln Asn Ala Ala Asn Lys Gly Val Ile Val Pro Lys Tyr Ser
ACA AGU GGG AAA UUC UUC AGU GGC UUG UUG GAU GAU GAA GAA GAA 8135
Thr Ser Gly Lys Phe Phe Ser Gly Leu Leu Asp Asp Asp Asp Asp
GAA CCU CAG AAU GUG AAU AUG UUG AAC GAA GAG GAC AUU GAG GUA 8180
Asp Pro Gln Asn Val Asn Met Leu Asn Asp Glu Asp Ile Glu Val
GAU AAG CGA AUG UUU GAG AAG AUU UCU GAG GUU AUA AGC GUG AUU 8225
Asp Lys Arg Met Phe Glu Lys Ile Ser Glu Val Ile Ser Val Ile
CAA CCC AGA AAG AAU GAG CUG GAA AGA AUG AUU GAG GAA GGC GUA 8270
Gln Pro Arg Lys Asn Glu Leu Asp Arg Met Ile Glu Asp Gly Val
CAC CAC AAG GUC GUA AAG CAG GCA AGG GUU AAC GAC AAG GGC UUA 8315
His His Lys Val Val Lys Gln Ala Arg Val Asn Asp Lys Gly Leu
GCC AAA GAC CCC AAC AUG GUG ACU AUC UUG ACG GAC AAA UUA AUU 8360
Ala Lys Asp Pro Asn Met Val Thr Ile Leu Thr Asp Lys Leu Ile
AAU AUU AGU GCG GUG AUC GUC AAU UUA ACG CCG ACA CGC CGG GCA 8405
Asn Ile Ser Ala Val Ile Val Asn Leu Thr Pro Thr Arg Arg Ala
UAC AUG AAC GUG GUA CGU CUU AUA GGC ACU AUA GUU GUU UGC CCA 8450
Tyr Met Asn Val Val Arg Leu Ile Gly Thr Ile Val Val Cys Pro
GCC CAC UAC UUG GAA GCU UUA GAG GAA GGA GAU GAG CUG UAU UUC 8495
Ala His Tyr Leu Asp Ala Leu Glu Asp Gly Asp Glu Leu Tyr Phe
AUU UGC UUC UCA UUG GUU AUC AAG CUC ACU UUU GAU CCA AGU AGA 8540
Ile Cys Phe Ser Leu Val Ile Lys Leu Thr Phe Asp Pro Ser Arg
GUG ACU CUC GUG AAU AGC CAG CAG GAU UUG AUG GUU UGG GAU CUU 8585
Val Thr Leu Val Asn Ser Gln Gln Asp Leu Met Val Trp Asp Leu
GGG AAC AUG GUA CCA CCC UCA AUU GAU ACU CUU AAA AUG AUA CCU 8630
Gly Asn Met Val Pro Pro Ser Ile Asp Thr Leu Lys Met Ile Pro
ACG CUU GAA GAC UGG GAU CAC UUU CAG GAU GGA CCA GGA GCC UUU 8675
Thr Leu Asp Asp Trp Asp His Phe Gln Asp Gly Pro Gly Ala Phe
GCU GUU ACG AAA UAU AAC UCG AAA UUC CCA ACC AAU UAU AUC AAC 8720
Ala Val Thr Lys Tyr Asn Ser Lys Phe Pro Thr Asn Tyr Ile Asn
ACA CUG ACU AUG AUU GAG AGG AUU AGG GCA AAU ACU CAG AAU CCC 8765
Thr Leu Thr Met Ile Glu Arg Ile Arg Ala Asn Thr Gln Asn Pro
ACG GGU UGU UAU UCC AUG AUG GGC UCC CAA CAU ACA AUC ACC ACA 8810
Thr Gly Cys Tyr Ser Met Met Gly Ser Gln His Thr Ile Thr Thr
GGA UUG CGA UAU CAA AUG UUC UCU CUU GAU GGA UUC UGC GGU GGG 8855
Gly Leu Arg Tyr Gln Met Phe Ser Leu Asp Gly Phe Cys Gly Gly
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UUA AUC CUG AGA GCC AGC ACA AAC AUG GUG AGA AAG GUC GUC GGG 8900
Leu Ile Leu Arg Ala Ser Thr Asn Met Val Arg Lys Val Val Gly
AUC CAC GUU GCU GGA AGC CAG AAU CAC GCU AUG GGA UAU GCA GAG 8945
Ile His Val Ala Gly Ser Gln Asn His Ala Met Gly Tyr Ala Glu
UGC CUU AUU GCA GAA GAU UUA CGG GCU GCA GUG GCG AGA UUG GCG 8990
Cys Leu Ile Ala Asp Asp Leu Arg Ala Ala Val Ala Arg Leu Ala
CUA GAU CCU AGA AGC ACC AUC CAG GCA AGU CUG AAA GGU AGG AUU 9035
Leu Asp Pro Arg Ser Thr Ile Gln Ala Ser Leu Lys Gly Arg Ile
GAU GCU GUU UCU AAA CAA UGU GGU UUA GAC AGA GCU CUG GGU ACG 9080
Asp Ala Val Ser Lys Gln Cys Gly Leu Asp Arg Ala Leu Gly Thr
AUA GGA UGU CAC GGG AAA GUU GCC UCU GAA GAU AUU ACA AGU GCC 9125
Ile Gly Cys His Gly Lys Val Ala Ser Asp Asp Ile Thr Ser Ala
GCC ACG AAA ACU UCC AUA AGA AAG UCA AGA AUA CAU GGU CUA GUG 9170
Ala Thr Lys Thr Ser Ile Arg Lys Ser Arg Ile His Gly Leu Val
GGU GAG AUU AGA ACU GAG CCU UCA AUU UUA CAC GCU CAU GAU CCC 9215
Gly Glu Ile Arg Thr Glu Pro Ser Ile Leu His Ala His Asp Pro
CGA CUG CCU AAA GAC AAG AUU GGG AAA UGG GAC CCG GUU AUU GAG 9260
Arg Leu Pro Lys Asp Lys Ile Gly Lys Trp Asp Pro Val Ile Glu
GCA UCA AUG AAG UAU GGU UCG AGA AUC ACA CCG UUC CCU GUA GAC 9305
Ala Ser Met Lys Tyr Gly Ser Arg Ile Thr Pro Phe Pro Val Asp
CAA AUU CUG GAA GUG GAG GAU CAU CUU UCU AAA AUG UUG GCC AAU 9350
Gln Ile Leu Asp Val Glu Asp His Leu Ser Lys Met Leu Ala Asn
UGU GAG AAU UCA AAA AAC AAG CGG CAG GUU AAU AAU CUA GAA AUA 9395
Cys Glu Asn Ser Lys Asn Lys Arg Gln Val Asn Asn Leu Asp Ile
GGG AUU AAU GGA AUU GAC CAG UCG GAU UAU UGG CAA CAG AUA GAA 9440
Gly Ile Asn Gly Ile Asp Gln Ser Asp Tyr Trp Gln Gln Ile Asp
AUG GAU ACU UCA AGU GGU UGG CCA UAC GCU AAG CGU AAA CCU GUU 9485
Met Asp Thr Ser Ser Gly Trp Pro Tyr Ala Lys Arg Lys Pro Val
GGG GCA GCU GGA AAG AAA UGG CUA UUC GAG CAA GAC GGC ACA UAU 9530
Gly Ala Ala Gly Lys Lys Trp Leu Phe Glu Gln Asp Gly Thr Tyr
CCC UCC GGA AAA CCU CGA UAU GUA UUU GGA GAU GCC GGG UUG AUU 9575
Pro Ser Gly Lys Pro Arg Tyr Val Phe Gly Asp Ala Gly Leu Ile
GAG AGC UAU AAC UCG AUG CUU GGU GAG GCG AAG CAA GGC AUU-AGU 9620
Glu Ser Tyr Asn Ser Met Leu Gly Glu Ala Lys Gln Gly Ile Ser
CCC ACU GUC GUC ACA AUU GAG UGC GCA AAA GAU GAG AGG CGG AAG 9665
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Pro Thr Val Val Thr Ile Glu Cys Ala Lys Asp Glu Arg Arg Lys
CUU AAU AAG AUA UAU GAG AAA CCC GCC ACU CGG ACG UUC ACC AUA 9710
Leu Asn Lys Ile Tyr Glu Lys Pro Ala Thr Arg Thr Phe Thr Ile
CUG CCA CCU GAG AUU AAU AUU UUA UUC AGG CAG UAU UUC GGA GAU 9755
Leu Pro Pro Glu Ile Asn Ile Leu Phe Arg Gln Tyr Phe Gly Asp
UUU GCA GCG AUG GUA AUG ACA UGU AGA GCC AAG CUU UUC UGU CAA 9800
Phe Ala Ala Met Val Met Thr Cys Arg Ala Lys Leu Phe Cys Gln
GUU GGC AUC AAC CCA GAG UCA AUG GAG UGG GGU GAU CUC AUG CUA 9845
Val Gly Ile Asn Pro Glu Ser Met Glu Trp Gly Asp Leu Met Leu
GGU CUA AAG GAG AAA UCA ACU AAG GGA UUU GCA GGA GAU UAU UCG 9890
Gly Leu Lys Glu Lys Ser Thr Lys Gly Phe Ala Gly Asp Tyr Ser
AAG UUC GAU GGA AUC GGA GAC CCC CAG AUU UAU CAU UCA AUU ACC 9935
Lys Phe Asp Gly Ile Gly Asp Pro Gln Ile Tyr His Ser Ile Thr
CAA GUA GUC AAC AAC UGG UAU AAC GAU GGG GAA GAA AAU GCG ACU 9980
Gln Val Val Asn Asn Trp Tyr Asn Asp Gly Asp Asp Asn Ala Thr
AUC AGG CAU GCU CUG AUA AGU AGC AUU AUA CAC AGG CGG GGC AUU 10025
Ile Arg His Ala Leu Ile Ser Ser Ile Ile His Arg Arg Gly Ile
GUG AAA GAA UAU UUG UUC CAG UAU UGC CAG GGU AUG CCA UCA GGG 10070
Val Lys Asp Tyr Leu Phe Gln Tyr Cys Gln Gly Met Pro Ser Gly
UUC GCC AUG ACA GUG AUA UUC AAU UCG UUU AUG AAC UAU UAU UAU 10115
Phe Ala Met Thr Val Ile Phe Asn Ser Phe Met Asn Tyr Tyr Tyr
CUG UCU UUG GCC UGG AUG AAU CUG AUA AGU GCA UCC CCC CUU AGU 10160
Leu Ser Leu Ala Trp Met Asn Leu Ile Ser Ala Ser Pro Leu Ser
CCA CAA GCU UCU UUG AGA UAU UUU GAU GAG UAU UGU AAG GUC AUU 10205
Pro Gln Ala Ser Leu Arg Tyr Phe Asp Glu Tyr Cys Lys Val Ile
GUU UAC GGU GAU GAU AAU AUU GUU GCC GUC AAC GAA GAA UUC UUA 10250
Val Tyr Gly Asp Asp Asn Ile Val Ala Val Asn Asp Asp Phe Leu
GAG UAC UAU AAC UUG AGG CUU GUG GCA GGC UAU CUU AGU CAA UUU 10295
Glu Tyr Tyr Asn Leu Arg Leu Val Ala Gly Tyr Leu Ser Gln Phe
GGA GUA AGC UAC ACU GAU GAC GCC AAG AAC CCA AUA GAG AAG AGC 10340
Gly Val Ser Tyr Thr Asp Asp Ala Lys Asn Pro Ile Glu Lys Ser
GAA CGA UAU GUG AAG AUA GAA GAC GUU ACG UUC UUA AAA CGG CGA 10385
Asp Arg Tyr Val Lys Ile Asp Asp Val Thr Phe Leu Lys Arg Arg
UGG GUG AGU CUU GGC GGU AGA GCU UCG AUG CUG UAC AAA GCU CCG 10430
Trp Val Ser Leu Gly Gly Arg Ala Ser Met Leu Tyr Lys Ala Pro
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CUU GAC AAG GUU AGC AUU GAG GAA AGG CUU AAC UGG AUC AGA GAG 10475
Leu Asp Lys Val Ser Ile Glu Asp Arg Leu Asn Trp Ile Arg Glu
UGU GAC GAU GGG GAA CUA GCU CUG GUG CAG AAC AUU GAA AGU GCU 10520
Cys Asp Asp Gly Asp Leu Ala Leu Val Gln Asn Ile Asp Ser Ala
CUG UAC GAA GCU AGU AUU CAU GGC CAC ACA UAU UUU GGA GAG CUU 10565
Leu Tyr Asp Ala Ser Ile His Gly His Thr Tyr Phe Gly Glu Leu
AAA GAU AAA AUU GCU AAA GCC UGU GAU GCA GUC AUG AUA ACU AUG 10610
Lys Asp Lys Ile Ala Lys Ala Cys Asp Ala Val Met Ile Thr Met
CCA AAU AUA AGA UAU AUU GAC UGC CAG AGA CGA UGG UGG ACC UCC 10655
Pro Asn Ile Arg Tyr Ile Asp Cys Gln Arg Arg Trp Trp Thr Ser
AUG ACU GGU GGG UAU CUU GAG CCG UCU GAU GUC ACC AAA CUU GUA 10700
Met Thr Gly Gly Tyr Leu Glu Pro Ser Asp Val Thr Lys Leu Val
AGG CUU GUU GAG AAA GGA CUA CUA GAC CCG AAA UCA GUA UGG AAA 10745
Arg Leu Val Glu Lys Gly Leu Leu Asp Pro Lys Ser Val Trp Lys
GAC CCA UUG UAC AGA ACC AAC AAG UUG CUA UUC GAC CUA UUG AGG 10790
Asp Pro Leu Tyr Arg Thr Asn Lys Leu Leu Phe Asp Leu Leu Arg
GAG GUU AAG GCA GCA CCC CUG GCC GCA UUU GUG GUC UAA 10829
Glu Val Lys Ala Ala Pro Leu Ala Ala Phe Val Val Stop
GUUACCCUUC UGACAAAAGG GCCUUGAACG GUUAUGGUUG AACAGAACUG 10879
UAAAAGGUGA GGACUAUAUA AGUUGUAGUA CGGAUGAGAU UGAAAGAAAA 10929
UUGGGUCACU CCCAUUCCUU UAUUAGGAAG GAGUGAUACC UUUUGUGUAG 10979
AUCUCUACCC CGAAACUCUU GAACCCUCAC ACGUUUUGGA GUAACCAGUA 11029
CACCCUUUUA GGUGGACCCU CGACUAUAGA UCGAGACCAA GUAUUGACUU 11079
GGUGUUCACG UCUUGCCGGA CGCAAAAUGG CACCCUUGUU UAGUGAUAUC 11129
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CCGGGGGAAA GAGAACGCCA GUUCUUUAAG CCAUAACUCU AGUGAGUUGA 11429
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CACGGGUUUG UGCUGCAGUA UUAAAUAUCA UUAAGGUACU GUGCUAUAGC 11679
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GGAGAAAUUA CAAAGCGUUG AACACAUUGA CGAUGGGGCC CAAUGCGCAC 11729
CCGGAUGUGU UACGCACCGU UUUUCUCUGU GUCACUAUAG AUAAAAGUGG 11779
GGUAGC-polyA 11785

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 bases

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: viral RNA

(A) DESCRIPTION: RNA codons for first 15 amino acids at 5' end of MCDV coat protein 1 (CP1)

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GUU UCA UUG GGU CGG UCA UUU GAG AAU GGA GUG CUU AUU GGU AGU 45
Val Ser Leu Gly Arg Ser Phe Glu Asn Gly Val Leu Ile Gly Ser

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(A) DESCRIPTION: first 15 amino acids of MCDV coat protein 3

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Leu Gln Val Ala Ser Leu Thr Asp Ile Gly Asp Leu Ser Ser Val 15

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(A) DESCRIPTION: first 15 amino acids of MCDV coat protein 1

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Val Ser Leu Gly Arg Ser Phe Glu Asn Gly Val Leu Ile Gly Ser 15

WHAT IS CLAIMED IS:

1. A DNA clone coding substantially solely for a coat protein of maize dwarf mosaic virus.

2. An expression cassette comprising a DNA clone according to Claim 1, operably linked to plant regulatory sequences which cause the expression of the DNA clone in plant cells.

3. An expression cassette comprising a DNA clone according to Claim 1, operably linked to bacterial expression regulatory sequences which cause the expression of the DNA clone in bacterial cells.

4. Bacterial cells containing as a foreign plasmid at least one copy of an expression cassette according to Claim 3.

5. Transformed plant cells containing as foreign DNA at least one copy of the DNA sequence of an expression cassette according to Claim 2.

6. Transformed cells according to Claim 5, further characterized in being cells of a monocotyledonous species.

7. Transformed cells according to Claim 6, further characterized in being maize, sorghum, wheat or rice cells.

8. Transformed cells according to Claim 5, further characterized in being cells of a dicotyledonous species.

9. Transformed cells according to Claim 8, further characterized in being soybean, alfalfa, tobacco or tomato cells.

10. A maize cell or tissue culture comprising cells according to claim 7.

11. A transformed maize plant, the cells of which contain as foreign DNA at least one copy of the DNA sequence of an expression cassette according to Claim 2.

12. A method of imparting resistance to maize chlorotic dwarf virus and maize dwarf mosaic virus - A to plants of a MCDV or MDMV-A susceptible taxon, comprising the steps of:

a) culturing cells or tissues from at least one plant from the taxon,

b) introducing into the cells of the cell culture or tissue culture at least one copy of an expression cassette comprising a DNA clone from the RNA genome of MCDV which codes substantially solely for the coat protein of the virus, operably linked to plant regulatory sequences which cause the expression of the DNA clone in the cells, and

c) regenerating MCDV-resistant whole plants from the cell culture or tissue culture.

13. A method according to Claim 12 which comprises the further step of sexually or clonally reproducing the whole plants in such manner that at least one copy of the sequence provided by the expression cassette is present in the cells of progeny of the reproduction.

14. A method according to Claim 12 in which the expression cassette is introduced into the cells by electroporation.

15. A method according to Claim 12 in which the expression cassette is introduced into the cells by microparticle bombardment.

16. A method according to Claim 12 in which the expression cassette is introduced into the cells by microinjection.

17. A method according to Claim 13 for providing MCDV and MDMV-A resistance in *Agrobacterium tumefaciens*-susceptible dicotyledonous plants in which the expression cassette is introduced into the cells by infecting the cells with *Agrobacterium tumefaciens*, a plasmid of which has been modified to include the expression cassette.

18. A method of imparting resistance to maize chlorotic dwarf virus and maize dwarf mosaic virus strain A to plants of a MCDV or MDMV-A susceptible taxon, comprising the steps of:

a) selecting a fertile, MCDV resistant plant prepared by the method of Claim 12 from a sexually compatible taxon;

b) sexually crossing the MCDV resistant plant with a plant from the MCDV susceptible taxon;

c) recovering reproductive material from the progeny of the cross; and

d) growing resistant plants from the reproductive material.

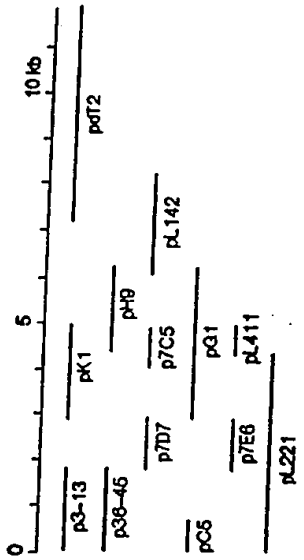
19. A method according to Claim 18 which comprises the further steps of repetitively:

a) backcrossing the MCDV resistant progeny with MCDV susceptible plants from the susceptible taxon; and

b) selecting for expression of MCDV resistance among the progeny of the backcross,

until the desired percentage of the characteristics of the susceptible taxon are present in the progeny along with MCDV resistance.

20. A DNA molecule coding for maize chlorotic dwarf virus or a portion thereof which is capable of conferring resistance to maize chlorotic dwarf virus when expressed in a plant cell.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pdT2
pL221

Figure 1

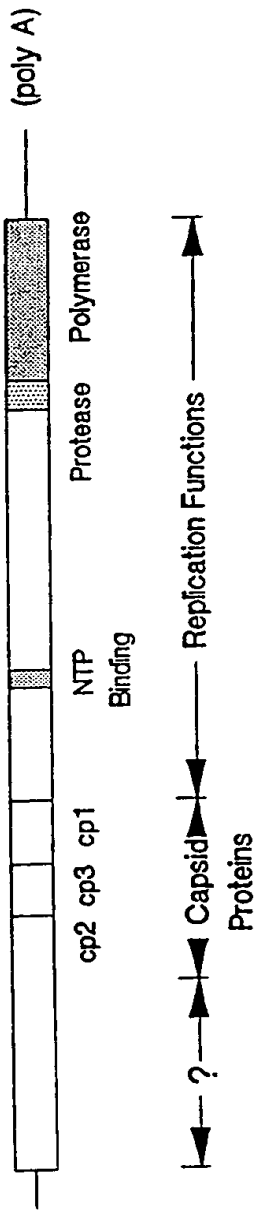


Figure 2

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08/038,768 24 March 1993 (24.03.93) US

(71) Applicants: PIONEER HI-BRED INTERNATIONAL, INC. [US/US]; 700 Capital Square, 400 Locust Street, Des Moines, IA 50309 (US). THE UNITED STATES OF AMERICA as represented by THE SECRETARY, UNITED STATES DEPARTMENT OF AGRICULTURE [US/US]; 12th and Independence Avenue. S.W., Washington, DC 20250-1400 (US).

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(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

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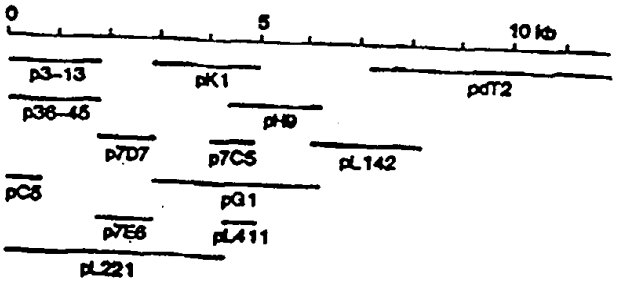
(54) Title: MAIZE CHLOROTIC DWARF VIRUS AND RESISTANCE THERETO

(57) Abstract

Methods and materials are provided to isolate the coat protein genes from maize chlorotic dwarf virus. One or more of these genes (MCDV-$CP_1$, MCDV-$CP_2$ or MCDV-$CP_3$) is then incorporated in an expression cassette designed for suitable expression in a plant cell system. The resulting transformation vector is then introduced into maize to provide cross protection to MCDV or related viral infections.



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# INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 94/03028

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/40 C12N15/82 C12N5/10 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| Y | see abstract 689<br>---<br>-/-- | 2,5-11 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 31 August 1994 | 21 -09- 1994 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. ( + 31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax ( + 31-70) 340-3016 | Maddox, A |

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| X | CHEMICAL ABSTRACTS, vol. 115, no. 11, 1991, Columbus, Ohio, US; abstract no. 108765, FRENKEL, M.J., ET AL. 'Unexpected sequence diversity in the amino-terminal ends of the coat proteins of strains of sugarcane mosaic virus' see abstract & J. GEN. VIROL. vol. 72, no. 2 , 1991 pages 237 - 242 | 1 |
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| X | --- CHEMICAL ABSTRACTS, vol. 115, no. 11, 1991, Columbus, Ohio, US; abstract no. 107314, JILKA, J.M. 'Cloning and characterization of the 3' terminal regions of RNA from select strains of maize dwarf mosaic virus and sugarcane mosaic virus' page 210 ; see abstract & PHD THESIS, UNIVERSITY OF ILLINOIS, URBANA, ILLINOIS, USA. 1990 & DISS. ABSTR. INT. B. vol. 51 , 1991 , 12 PART 1 page 5719 | 1 |
| X | --- DATABASE BIOSIS BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US BR38:57628 GE, X., ET AL. 'Characterization of maize chlorotic dwarf virus MCDV rna' see abstract & PHYTOPATHOLOGY vol. 79, no. 10 , 1989 page 1157 | 20 |
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| P,X | WO,A,93 14210 (SANDOZ) 22 July 1993<br>see the whole document | 1,2,5-11 |
| A | DATABASE BIOSIS<br>BIOSCIENCES INFORMATION SERVICE,<br>PHILADELPHIA, PA, US<br>BR38:57625<br>MAROON, C.M., ET AL. 'Serological relationships of the capsid proteins of the type isolate of maize chlorotic dwarf virus MCDV-T'<br>see abstract<br>& PHYTOPATHOLOGY<br>vol. 79, no. 10 , 1989<br>page 1157 | 1-20 |
| A | CHEMICAL ABSTRACTS, vol. 115, no. 7, 1991, Columbus, Ohio, US;<br>abstract no. 65640,<br>GE, X. 'Characterization of the genome of maize chlorotic dwarf virus and an associated satellite virus'<br>see abstract<br>& Dissertation available , univ. microfilms int.,<br>order no. DA 9105112<br>Diss. abstr. int. B 51(10), 4666. | 1-20 |
| A | DATABASE BIOSIS<br>BIOSCIENCES INFORMATION SERVICE,<br>PHILADELPHIA, PA, US<br>BR46:293<br>KOONIN, E.V., ET AL. 'Evolution and taxonomy of positive-strand RNA viruses: Implications of comparative analysis of amino acid sequence'<br>see abstract<br>& CRITICAL REVIEWS IN BIOCHEMISTRY AND MOLECULAR BIOLOGY<br>vol. 28, no. 5 , 1993<br>pages 375 - 430 | 1-20 |
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...information on patent family members

Inter ...nal Application No
PCT/US 94/03028

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
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| | | AU-A- | 6452886 | 30-04-87 |
| | | JP-A- | 62201527 | 05-09-87 |
| WO-A-9314210 | 22-07-93 | NONE | | |